(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 July 2005 (14.07.2005)

PCT

(10) International Publication Number
WO 2005/062865 A2

(51) International Patent Classification: Not classified

(21) International Application Number:
PCT/US2004/042997

(22) International Filing Date:
21 December 2004 (21.12.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/532,144 22 December 2003 (22.12.2003) US

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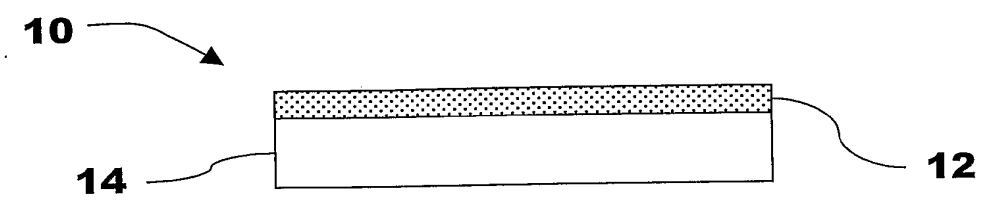
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: OPTICALLY ENCODED PARTICLES WITH GREY SCALE SPECTRA



(57) Abstract: The invention concerns a particle (10, 10a) having a grey scale code embedded in its physical structure by refractive index changes between different regions of the particle.

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OPTICALLY ENCODED PARTICLES WITH GREY SCALE SPECTRA

TECHNICAL FIELD

A field of the invention is encoding. Additional exemplary fields of the invention include the life sciences, security, product marking, food processing, agriculture, and chemical detection.

BACKGROUND ART

A well-appreciated need for labeling exists in society. Labeling is a fundamental basis for tracking and identifying. Encoding can be used as a form of labeling understood by persons or equipment, as in the case of bar coding. At the microscale, however, labeling/encoding itself becomes difficult.

Strategies to encode microscale materials have accordingly received increased attention for such uses as high-throughput screening in the fields of drug discovery, genetics screening, biomedical research, and biological and chemical sensing. Concurrent research strategies for measuring an increased number of analytes while minimizing the necessary sample volume have focused on either on-chip spatially differentiated arrays or encoded beads. Large arrays have been developed for biological and/or chemical sensing purposes by making use of positional encoding to register specific analyte responses. The main advantage of using an array over a conventional single analyte sensor is the ability to process and analyze a large number of analytes simultaneously. Positional arrays, however, can suffer from slow diffusion rates and limits on the concentration ranges of analytes being sensed. An alternative approach is to use individually encoded beads.

Early attempts to encode particles used fluorescent or infrared-active molecules as binary markers. More recently, cadmium selenide quantum

dots have been demonstrated as viable candidates for particle encoding based on their unique fluorescent properties. Quantum dots have the advantage over organic molecules of increased stability towards photobleaching, sharper fluorescence peaks, improved solubility characteristics, and large excitation frequency ranges. With six colors (limited to the peak width of the fluorescence in the visible range) and ten intensity levels, 10^6 particles could theoretically be encoded. In practice, this number is difficult to obtain because of spectral overlap and sample inhomogeneities. Also, despite the increased photostability of quantum dots, fluorescence quenching is still possible, casting uncertainty on using relative intensity measurements as a reliable encoding method.

Another encoding strategy has used sub-micron metallic rods. The sub-micron metallic rods are prepared by electrodeposition of metals on a porous membrane in alternating strips of controlled thickness. Different reflection characteristics of the various metals are used as a barcode for identification purposes. Reflection spectroscopy does not have the disadvantage of photobleaching inherent with fluorophores. Additionally, fluorescent analytes do not interfere with the particle signal. Deposition of rods is a relatively complex process, however, and may be difficult to apply as an encoding strategy where, for example, a large number of codes is desirable because each rod must be brought into focus in an optical reader (such as a microscope) in order to read out the codes. There remains a need for encoding strategies at the microscale.

DISCLOSURE OF THE INVENTION

The invention concerns a particle having a grey scale code embedded in its physical structure by refractive index changes between different regions of the particle.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic diagram of a multi-layer encoded particle of the invention; and

FIG. 2 illustrates a preferred embodiment method of fabricating encoded particles.

BEST MODE OF CARRYING OUT THE INVENTION

The invention concerns a particle having a grey scale code embedded in its physical structure by refractive index changes between different regions of the particle. A change in the refractive index is preferably obtained by varying porosity formed in the particle. Reflections taken from the particles produce an optical signature, in the visible and/or non-visible wavelengths. In preferred embodiments, the number of peaks, their locations, and intensities can be used to produce a high number of unique optical signatures exhibiting grey scale codes. In preferred embodiment formation methods, a porous encoded structure is produced by an etching process during which the etching conditions are varied during pore formation according to a computer generated waveform designed to produce a grey scale coding. A dicing may be conducted to form individual encoded particles having a range of small sizes, e.g., from hundreds of nanometers to hundreds of microns.

Methods and particles of the invention are applicable to a variety of industries, including but not limited to drug discovery, biological screening, chemical screening, biological labeling, chemical labeling, *in vivo* labeling, security identification and product marking. Various attributes of the particles and methods of the invention enable a wide range of applications in various industries. The small size of the particles facilitates ready incorporation into various hosts, e.g., products, test kits, assays, powders (such as explosives for identification), pastes, liquids, glass, paper, and any other host or system that can accept small particles. *In vivo* detection is enabled by biocompatible particles of the invention, which may then be queried, for example, through tissues using near infrared and infrared wavelengths that penetrate tissues.

In accordance with the aforementioned exemplary aspects and applications of the inventions, preferred embodiment particles are identified by the grey scale code inherent to the reflectivity spectrum of their varying porous structure. In another aspect of the invention, matter, e.g., biological or chemical matter, is hosted by the porous structure and the particle becomes a tag identifying the matter hosted by the pores. In another aspect of the invention, a variance in the reflectivity spectrum of an encoded particle can indicate the presence, absence or quantity of matter within the particle's pores.

Referring to FIG. 1, a preferred embodiment encoded particle 10 is shown in cross-section. The encoded particle 10 includes a porous thin film 12. The porous thin film 12 having varying porosity is shown in FIG. 1 as being formed on a substrate 14. However, embodiments of the invention include particle structures released from a substrate upon or from which they were initially formed. The thin film 12 is encoded to produce an interference pattern in the reflectivity spectrum that forms an optical signature including a grey scale code. Particles 10 of the invention may be specifically encoded by controlling etching conditions according to a computer generated waveform during formation of the particle 10.

The porous thin film 12 may be formed of any porous semiconductor or insulator. In preferred embodiment particles of the invention, porous silicon is used to form the thin film 12. Controlled anodic etching of crystal silicon in hydrofluoric acid solution permits control of both the porosity and thickness of porous thin film 12 The time of etching controls the thickness of a porous layer, while the etching current density controls the porosity. The thicknesses and porosities of thin films 12 are controlled in accordance with a computer generated waveform.

Porous silicon is a preferred material for the thin film 12. Porous silicon has a number of demonstrated advantages. For example, porous silicon has been demonstrated to be biocompatible. In addition, the surface chemistry of oxidized porous silicon is effectively that of silica. Accordingly, the surface

chemistry is well understood for biochemical derivatization and ligand immobilization.

In preferred embodiments, the thin film 12 is formed to include a receptor material within the porous structure. The purpose of the receptor is to bind a particular analyte of interest. Exemplary receptors (also referred to as binders) are disclosed, for example, in U.S. Patent No. 6,248,539 entitled "Porous Semiconductor Based Optical Interferometric Sensor". Receptor molecules may be adsorbed or otherwise associated with the porous silicon thin film 12 by any approach that leads to the tethering of the receptor molecules to the porous thin film 12. This includes, without limitation, covalently bonding the receptor molecules to the semiconductor, ionically associating the receptor molecules to the layers, adsorbing the receptor molecules onto the surface of the layers, or other similar techniques. Association can also include covalently attaching the receptor molecules to another moiety, which is in turn covalently bonded to the porous thin film 12, or binding the target molecule via hybridization or another biological association mechanism to another moiety which is coupled to the porous thin film 12. Specific additional examples include receptor ligands that have been attached to porous silicon layers to produce biosensors. An analyte bound to a particle 10 of the invention becomes identifiable and traceable by the encoding provided by the particle 10.

We have demonstrated reproducibility in the spectral position and grey level (spectral height) by using waveform and spectra design in accordance with equations 1-4:

$$A_n = (A_{nmax} - A_{nmin})/2 \quad (1)$$

$$k_n = \text{frequency} = 1/\text{period} \quad (2)$$

$$y_n = A_n [\sin(k_n t - \Phi) + 1] + A_{nmin} \quad (3)$$

$$y_{comp} = [y_1 + \ldots + y_n]/n \quad (4)$$

Equation (1) defines the amplitude of sine component n, which results in the spectral peak height, or grey scale of a bit. Equation (2) defines the frequency of the each sine component, which results in the spectral position of a peak, or indentification of a bit (1st bit, 2nd bit, etc....). Equation (3) defines sine

component n. Equation (4) defines the composite waveform used to drive the electrochemical etch. The spectral peak position is a function of the frequency, k, of the sine component y(t) of the time domain. Spectral peak position and position in k-space are synomomous and related by c=wavelength*k(frequency). Grey levels in the heights of the spectral lines can be determined based on each sine components' amplitude. Fourier analysis may be used as a modeling tool to approximate the spectra of the resulting photonic crystal in advance of the etching conducted to create the porosity pattern. The formation of a composite waveform may be achieved by the addition of two separate sine components in accordance with equation (4). In a porous silicon photonic crystal, the average amplitude of a composite waveform must stay the same if the spectral line group is to maintain the same absolute spectral position after a change in one or more of the sine components' amplitude. Consider, for example, two waveforms, wf_1 and wf_2, correspond to two codes of the form: Acomp (bit 1) = (A1 + . . . +An)/n; Acomp (bits 2. . .n) = {[A1 - (x/(n-1))] + . . . + [An + x]}/n. If the second code has amount x added to the amplitude of its sine component, then the following condition must be met: Acomp(wf-1)=Acomp(wf_2), where Acomp is the amplitude of the composite waveform formed from the addition of wf_1 and wf_2. Resultant k-space spectra will reveal that a constant position is maintained while amplitudes of sine components are changed with the above equations. When incident white light strikes the encoded film, only light containing frequencies that match the spatial frequencies of varying porosity present in the film are reflected back. This is a natural optical transform which is much like a Fourier transform.

Experiments were conducted to demonstrate the invention. Grey scale samples were prepared by anodically etching p++ type, B-dope, (100) oriented silicon with <1mOhm-cm resistivity in a solution of 3:1 HF (48%, aq)/ethanol by volume. Computer generated anodic current waveforms consistent with the above explanations for grey scale coding were applied and a

platinum mesh electrode was used as the counter electrode. Results were consistent with expectations.

The intensity of peaks in the reflectance spectrum is controlled by the refractive index at interfaces between thin film 12, determined by a change in porosity between adjacent layers. Such change may be gradual or sharp. The position of peaks is controlled by adjusting layer thicknesses. Additional encoding is possible by variation of the relative intensities of each reflectivity peak, which can be engineered into particles 10 of the invention by adjustment of the electro chemical etch parameters to control porosity of the thin film 12. A particle 10 having of the invention encodes L^Ncodes, where N is the number of spectral lines and L is the number of grey levels possible in each spectral line.

Referring now to FIG. 2, a preferred method for forming an encoded porous particle 10 is shown. A suitable semiconductor or insulator, e.g., a silicon wafer, is selected for processing (step 14). For example, silicon wafers may be cut to size and be masked to have portions exposed for etching. An exemplary suitable silicon material is a single crystalline silicon wafer. Spatial encoding is then defined (step 16). The spatial encoding defines a range of codes over the material to be etched. Conducting a spatially resolved etch allows codes to be programmed in particle-sized sections of the wafer. An exemplary spatially resolved etch is disclosed in U.S. Patent No. 5,318,676, entitled "Photolithographic fabrication of luminescent images on porous silicon structures", published June 7, 1994. In an alternative process, the step of spatial defining (step 16) is omitted. For example, a single wafer or an area of wafer could be etched to include particles having a single code. In that case, other wafers could be etched to have particles having a different code. Anodic etching is then commenced, for example, in an aqueous solution of hydrofluoric acid and ethanol (step 18). Etching is then conducted with etching conditions varying according to the defined encoding strategy (step 20). A grey scale code or codes of the invention are etched into the wafer. The traverse (vertical direction in FIG. 1) encoded but still connected particles may

be lifted off from the silicon wafer (step 22), for example by a high level of electropolishing current. Areas between spatially defined etch sections may be cut to separate differently encoded wafer sections. Individual particles are then separated (step 24) in a dicing that may be conducted, for example, by mechanical agitation or ultrasonic fracturing. The particle separation (step 24) preferably produces micron-sized particles, e.g., particles in a range from a few hundred nanometers to a few hundred micrometers. A step of particle designation (step 26) may be conducted after the particle separation (step 24) or subsequent to step 20 or step 22. Particle designation may comprise, for example, chemical modification of the porous multi-layer structure 12_1-12_N for specific biological, biomedical, electronic, or environmental applications. As an example, the particles can be modified with a receptor for a desired analyte or with a targeting moiety (such as a sugar or a polypeptide). Additionally, binding can be signaled, for example, by fluorescence labeling of analytes or analyte autofluoresence. In use of particle 10, the particle can be identified by its optical signature upon binding of the designated targeted analyte. This step of designation may also be omitted in embodiments of the invention.

In other embodiments of the invention, encoded particles can be placed into a suitable hosts, namely any liquid, powder, dust, or other material that will hold encoded micron sized particles of the invention. Particles placed in hosts, for example, could be used to identify the source of a manufactured powder such as an explosive. Another potential host is an animal. Particles of the invention being biocompatible may be implanted *in vivo* into an animal host. The reflectivity spectrum of preferred embodiment porous silicon particles 10 of the invention, for example, encompasses the visible, near infrared, and infrared spectra. This presents the opportunity to sense the grey scale code of a particle of the invention through barriers such as living tissue.

A first example embodiment is stand-off detection. This is a chemical detection technique to identify an analyte from a distance. A particle 10 of the invention includes a receptor to sense a particular analyte. Both the grey scale code of the particle and an indication of binding of the analyte can

be detected in the reflectivity spectrum, for example, with use of a low power laser. The receptor, for example, can be specific to sense biomolecules or to attach the encoded particle to a cell, spore, or pollen particle.

Another preferred exemplary application of the invention is for biomolecular screening via the encoded particle 10 of the invention. Millions of grey scale codes are possible with a small number of layers. A simple antibody-based bioassay using fluorescently tagged proteins has been tested. Periodic Rugate style encoding was used as described above with respect to the exemplary chemical sensing embodiments. By masking the wafer before etching, well-defined slabs of particles may be generated.

The layered grey scale porous-silicon encoded structures offer several advantages over existing encoding methodologies. Porous-silicon encoded structures can be constructed that display features spanning the visible, near-infrared and infrared regions of the spectrum. Unlike encoding schemes based on stratified metallic nanorods, fluorescence or vibrational signatures, encoded particles of the invention can be probed using light diffraction techniques; thus it is not necessary to use imaging optics in order to read the codes. Encoded particles may be assayed using a conventional fluorescence tagging technique, and sensitive chemical and biochemical detection can also be built into the optical structure of the encoded particles, eliminating the need for fluorescent probes and focusing optics. In addition, because preferred embodiment oxidized porous-silicon encoded particles present a silica-like surface to the environment, they do not readily quench luminescence from organic chromophores, and they can be handled and modified using the chemistries developed for glass bead bioassays. Silicon-based encoded particles may be readily integrated with existing chip technologies.

The use of encoded silicon particles of the invention in medical diagnostic applications has advantages over organic dyes or quantum dots. *In vivo* studies have shown the biocompatibility of porous silicon, as well as the long-term stability of reflectance data from multilayer structures. Additionally,

the possibility of optically addressing particles at near-infrared, tissue-penetrating wavelengths without the losses associated with low fluorescence quantum yields makes these materials amenable to *in vivo* diagnostics. Finally, because the porous codes are an integral and orderly part of the porous structure, it is not possible for part of the code to be lost, scrambled or photobleached, as can occur with quantum dots or fluorescent molecules.

While specific embodiments of the present invention have been shown and described, it should be understood that other modifications, substitutions and alternatives are apparent to one of ordinary skill in the art. Such modifications, substitutions and alternatives can be made without departing from the spirit and scope of the invention, which should be determined from the appended claims.

Various features of the invention are set forth in the appended claims.

CLAIMS:

1. An optically encoded particle (10, 10a), comprising:

a layer of material; and

porosity within the layer of material configured to produce an interference pattern in the reflectivity spectrum that forms an optical signature including a detectable grey scale code.

2. The particle of claim 1, wherein the particle has a diameter of hundreds of microns or less.

3. The particle of claim 1, wherein said porosity is formed in accordance with an etching waveform, and there is a correspondence between sine components of the etching waveform and a spectral position and height of peaks in Fourier transformed k-space of said interference pattern.

4. The particle of claim 3, wherein said interference pattern in the reflectivity spectrum extends beyond the visible spectrum.

5. The particle of claim 3, wherein the height of the spectral peaks correspond to sine components' amplitudes.

6. The particle of claim 1, wherein said material comprises a semiconductor.

7. The particle of claim 6, wherein said semiconductor comprises silicon.

8. The particle of claim 1, wherein said first porous layer and said n additional porous layers are formed from an insulator.

9. The particle of claim 1, further comprising a receptor for binding a predetermined analyte.

10. An optically encoded particle (10, 10a), comprising a thin film in which porosity varies in a manner to produce an optical signature detectable in the reflectivity spectrum that when converted to Fourier k-space exhibits a grey scale code.

11. The particle of claim 10, further comprising a receptor.

12. The particle of claim 11, wherein said receptor is a receptor for a biological analyte.

13. The particle of claim 11, wherein said receptor is a receptor for a chemical analyte.

14. The particle of claim 11, wherein said receptor is a receptor for a gaseous analyte.

15. The particle of claim 10, further comprising a fluorescence tag for assaying the particle.

16. The particle of claim 10, wherein the thin film comprises porous silicon.

17. The particle of claim 10, being micron-sized.

18. A method for encoding thin films, comprising steps of:

etching a semiconductor or insulator substrate to form a thin film including pores;

varying etching conditions to vary porosity in the thin film according to a pattern that will generate an optical signature in the reflectivity spectrum in response to illumination, the optical signature including a grey scale code.

19. The method of claim 18, wherein said step of varying comprises applying an etching waveform formed by the addition of at least two separate sine components in accordance with $y_{comp} = [y_1 + \ldots + y_n]/n$, where y_n are the sine components.

20. The method of claim 18, wherein the grey scale code is revealed in naturally optically converted k-space.

21. The method according to claim 18, further comprising a step of separating the thin film from the semiconductor or insulator substrate.

22. The method according to claim 18, further comprising a step of separating the thin film into particles.

23. The method according to claim 18, further comprising a step of placing a particle within a host.

24. The method according to claim 18, further comprising steps of:

generating an interference pattern in the reflectivity spectrum by illumination of one or more of the particles;

determining a particle's code from the position and heights of peaks in k-space.

25. The method according to claim 18, wherein said step of varying etching conditions varies the etching conditions according to sine component equations.

26. The method according to claim 18, further comprising a step of spatially defining the semiconductor or insulator substrate to conduct said step of etching in a spatially defined location or locations.

27. The method according to claim 26, wherein said step of varying further varies etching conditions in different spatially defined locations to encode multiple codes in the thin film.

28. The method according to claim 27, further comprising a step of separating the thin film from the semiconductor or insulator substrate.

29. The method according to claim 28, further comprising a step of separating the thin film into particles.

30. A method for identification of an analyte bound to an encoded particle or identification of a host including an encoded particle of claim 10, the method comprising steps of:

associating the encoded particle with the analyte or the host;

generating an interference pattern in the reflectivity spectrum by illumination of the particle;

determining the particle's code from the interference pattern;

identifying the analyte or the host based upon said step of determining.

31. The method according to claim 30, further comprising a step of designating the particle to bind an analyte by modifying the particle with a specific receptor or targeting moiety.

32. The method according to claim 31, wherein the targeting moiety is a sugar or polypeptide.

33. The method according to claim 32, further comprising a step of signaling binding of an analyte by fluorescence labeling or analyte autofluorescence.

34. A method of encoding micron sized particles, the method comprising steps of:

etching a wafer to form a thin film having a varying porosity that will produce a detectable optical signature grey scale code in response to illumination;

applying an electropolishing current to the wafer to remove the porous film from the wafer;

dicing the film into micron-sized particles, each micron-sized particle maintaining an optical signature produced by said step of etching.

35. The method according to claim 34, further comprising a step of modifying the particles with a specific receptor or targeting moiety.

36. An encoded micron-sized particle (10, 10a) having a grey scale code embedded in its physical structure by refractive index changes between different regions of the particle.

37. The particle of claim 36, further comprising a receptor.

38. The particle of claim 37, wherein said receptor is a receptor for a biological analyte.

39. The particle of claim 37, wherein said receptor is a receptor for a chemical analyte.

40. The particle of claim 37, wherein said receptor is a receptor for a gaseous analyte.

41. The particle of claim 37, further comprising a fluorescence tag for assaying the particle

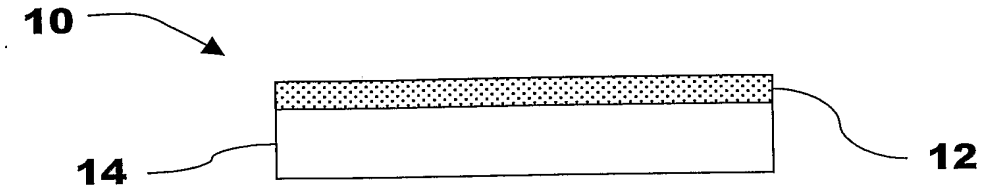

FIG. 1

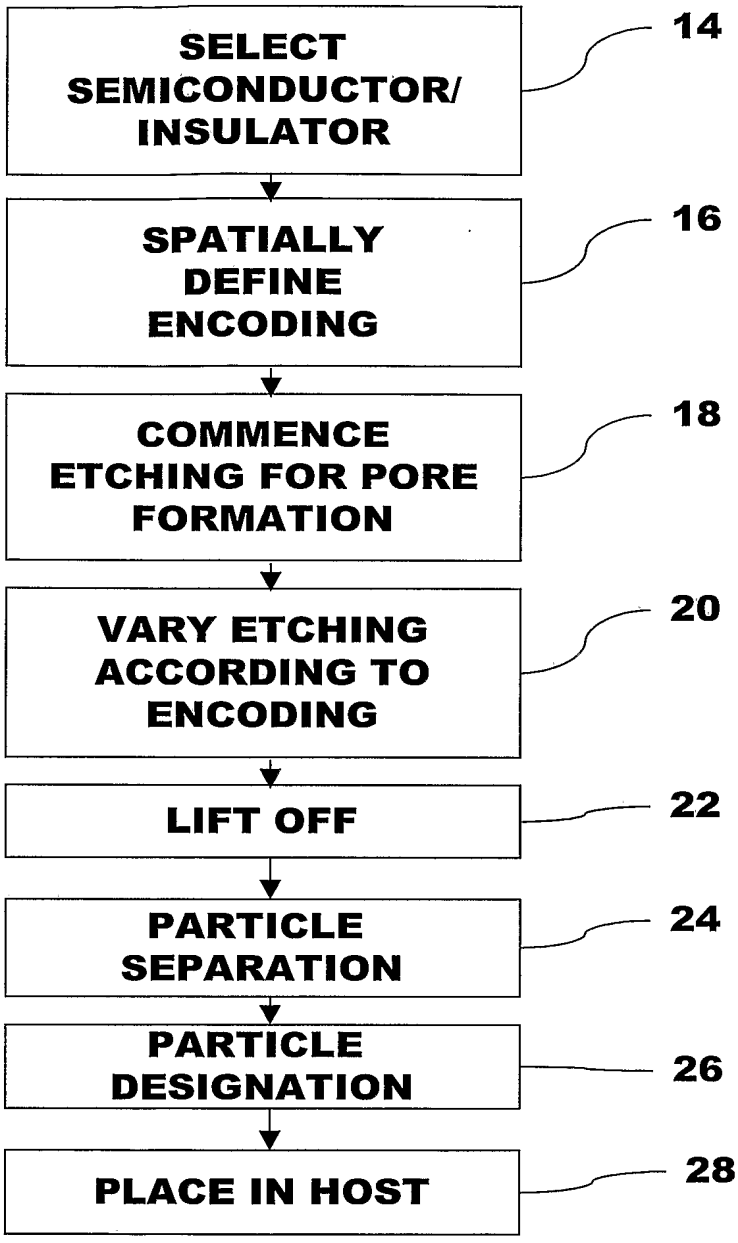
SELECT SEMICONDUCTOR/ INSULATOR
14
SPATIALLY DEFINE ENCODING
16
COMMENCE ETCHING FOR PORE FORMATION
18
VARY ETCHING ACCORDING TO ENCODING
20
LIFT OFF
22
PARTICLE SEPARATION
24
PARTICLE DESIGNATION
26
PLACE IN HOST
28

FIG. 2